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First record of bioluminescence in fungi from Mexico

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Abstract—Two species of bioluminescent fungi are recorded from Mexico. *Mycena stylobates* is characterized by a pubescent basal disc, pileus with a separable gelatinous pellicle, and cheilocystidia with coarse excrescences, and represents a new record for the country. *Panellus stipticus,* which is distinguished by having rounded flabelliform basidiomata and allantoid to elongate basidiospores, has been previously recorded from Mexico but without any observation of bioluminescence. The specimens were collected in relict cloud forests from the state of Veracruz, Mexico.

Key words—*Agaricales*, biodiversity, *Mycenaceae*, mycobiota, taxonomy

Introduction

Fungal bioluminescence results from a chemical reaction between a protein generically known as luciferin and an enzyme generically known as luciferase that produces emission of green light with a wavelength of 520–530 nm. Light can be emitted from the mycelium, or basidiome, or both (Desjardin et al. 2008, Shih et al. 2014, Chew et al. 2015). More than 80 macromycete species (all in *Basidiomycota*) have been recorded as bioluminescent. They are distributed in *Armillaria* (Fr.) Staude, *Favolaschia* (Pat.) Pat., *Filoboletus* Henn., *Gerronema* Singer, *Mycena* (Pers.) Roussel, *Neonothopanus* R.H. Petersen & Krisai, *Omphalotus* Fayod, *Panellus* P. Karst., *Poromycena* Overeem, and *Roridomyces* Rexer (Desjardin et al. 2008, Chew et al. 2015).

DNA based sequence analyses (Desjardin et al. 2008, Chew et al. 2015) group these genera in three lineages: 1) *Armillaria*, 2) mycenoid, and 3) *Omphalotus*. Bioluminescent macromycetes are found in tropical and temperate forests, and most of the species are saprobes that grow in high-moisture substrates such as decomposing trunks, roots, leaf-litter, and living tree bark. These fungi have been recorded from Brazil, Costa Rica, United States, Thailand, and numerous other countries (Corner 1950, 1954; Deheyn & Latz 2007; Desjardin et al. 2010; Chew et al. 2015; Seas-Carvajal & Avalos 2013). In Mexico, although genera such as *Mycena*, *Armillaria*, and *Panellus* have been recorded in taxonomic works or fungal lists (mainly from cloud forests; Chacón & Medel 1993), little attention has been given to bioluminescence. The aim of this work is to contribute to the knowledge of the biodiversity of bioluminescent macromycetes from Mexico.

Materials & methods

All specimens studied were collected in cloud forests and are deposited in the Mycological collection of the herbarium of Instituto de Ecología, Xalapa, Veracruz, Mexico (XAL). For the identification of macroscopic fungi, we followed Largent et al. (1977) and used the terminology for microstructures according to Vellinga (1988). For the microscopic analysis hand-made sections were made from different parts of dry basidiomata previously rehydrated in 70% ethyl alcohol, then mounted in a 5% solution of KOH, Melzer´s solution, or Congo red. At least 30 measurements were recorded for each taxonomically informative structure. Basidiospore shape was determined according to the Q quotient (length/width ratio). Basidiospores, cystidia, and other important microscopic structures were drawn using a drawing tube mounted on a Leica DME light microscope. All measurements represent the minimum and maximum dimensions observed, and measurements in parentheses represent dimensions that are considered outside the normal range. Photographs recording the bioluminescence in complete darkness were taken for *Mycena stylobates* with a Nikon D810 camera with a 105 mm macro lens (340 seconds exposure, ISO 6400, f/5.6 and f/6.3) and for *Panellus stipticus* with a Nikon D3200 camera with a 18-55 mm lens (60 seconds exposure, ISO 800, f/3.5).

Taxonomy

Mycena stylobates (Pers.) P. Kumm., Führ. Pilzk.: 108 (1871). Plates 1a-c; 2a-i

Pileus 3–13 mm diam, broadly parabolic, obtusely conical or hemispherical to convex or plano-convex, sometimes centrally depressed in older basidiomata; surface moist, subviscid, minutely squarrose, glabrous, translucent-striate to sulcate, pale brown at first to pale grey-brown with a whitish margin, or greyish white. Context thin, whitish. Lamellae adnexed, narrowly adnate, sometimes forming a pseudocollarium, white, close (14–18) with 2 series of lamellulae,

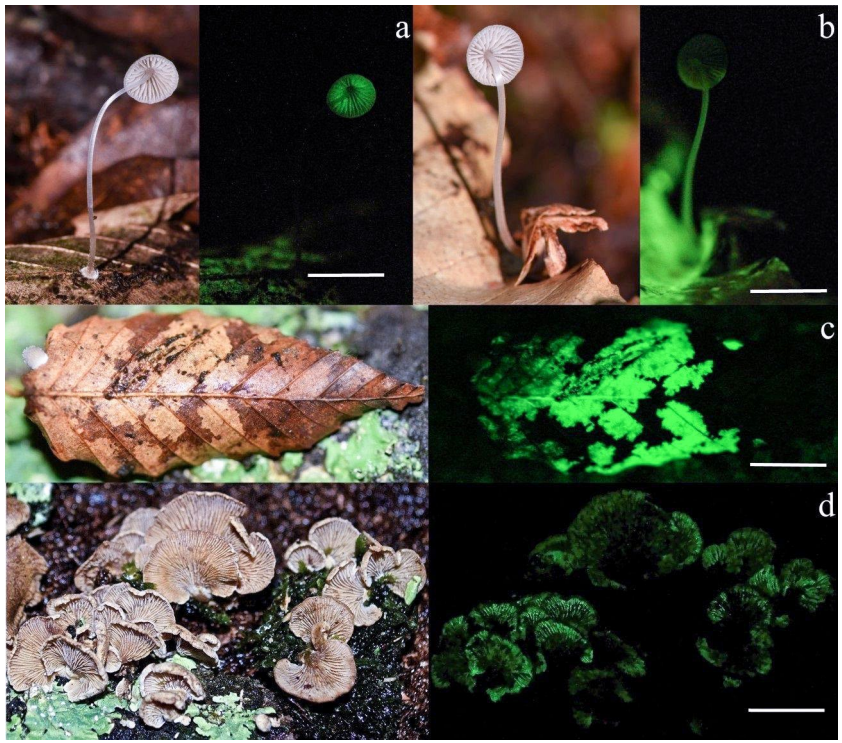

Plate 1. Bioluminescent Mexican basidiomes (paired light and dark exposures). ***Mycena stylobates*** (A = A. Cortés-Pérez & A. Rockefeller 1344; B, C = A. Cortés-Pérez & A. Rockefeller 1810). ***Panellus stipticus*** (D = A. Cortés-Pérez 1452). Scale bars = 10 mm.

edges even. Stipe 15–30 × 1–2 mm, central, equal, cylindrical, hollow, dry, pubescent, especially towards the apex, whitish and greyish white towards the base, emerging from a pubescent, sulcate, whitish basal disc. Basidiospores (6–)6.5–9 × 3–4.4(–4.8) µm (Q = 1.7–2.4), ellipsoid to oblong, smooth, hyaline, amyloid, thin-walled. Basidia 19–24 × 6–8 µm, clavate, 4-spored, hyaline, inamyloid. Cheilocystidia (20–)23–43(–50) × (6–)7–11(–13) µm, fusoid, clavate or cylindrical with one or several coarse excrescences with rounded apices, hyaline, inamyloid, forming a sterile lamellar edge. Pleurocystidia absent. Subhymenium cellular to inflated-ramose, hyphae 1.5–5 µm diam, hyaline, inamyloid. Lamellar trama regular, hyphae 1.5–21 µm diam, cylindrical, hyaline, dextrinoid. Pileipellis an ixocutis >60 µm thick, hyphae, 2–6 µm diam, the uppermost hyphae of the pileipellis covered with numerous,

cylindrical diverticula, around 2–8 × 0.5-1.5 µm. The lowermost hyphae of the separable pileipellis are smooth, cylindrical, hyaline, inamyloid. Pileus trama, hyphae 2–27 µm diam, inflated or cylindrical, hyaline, dextrinoid. Caulocystidia 34–82.5(–112) × 3–6.5(–8) µm, cylindrical with narrower rounded apices, in fascicles, hyaline, inamyloid. Basal disc cystidia 49–128 × 3–6(–8) µm, cylindrical with narrower rounded apex, hyaline, inamyloid. Clamp connections present.

Bioluminescence—Mycelium and occasionally in the pileus, or (rarely) in the entire basidiome.

Ecology & distribution—Solitary to scattered on leaf litter of *Fagus grandifolia* var. *mexicana* (Martínez) Little or *Quercus* spp. and on decaying twigs of ferns, in cloud forests between 1500–1900 m a.s.l.

Material studied—**MEXICO: Veracruz, Municipality of Acajete**, Mesa de la Yerba, 27 September 2014, leg. A. Cortés-Pérez 1205; 3 October 2014, leg. A. Cortés-Pérez 1214; 24 October 2014, leg. A. Cortés-Pérez 1223; 14 November 2014, leg. A. Cortés-Pérez 1244; 3 July 2015; leg. A. Cortés-Pérez & A. Rockefeller 1344; 18 September 2015, leg. A. Cortés-Pérez 1406; 27 September 2015, leg. A. Cortés-Pérez 1417; 20 August 2016, leg. A. Cortés-Pérez 1631; 2 September 2016, leg. A. Cortés-Pérez 1675; October 10, 2016, leg. A. Cortés-Pérez & A. Rockefeller 1810; **Municipality of Tlalnelhuayocan**, Los Capulines, 22 November 2014, leg. A. Cortés-Pérez 1254.

Comments—*Mycena stylobates* (*M.* sec. *Basipedes*) is characterized by a pileus with a separable gelatinous pellicle, claviform cheilocystidia with coarse excrescences, and a pubescent basal disc (Aronsen 2016). According to Aronsen (2016), *M. tenuispinosa* J. Favre is the most macroscopically similar species due to its pileus covered with small spinules; however the presence of cheilocystidia with thin excrescences separates the two species; Aronsen (2016) also cites *M. mucor* (Batsch) Quél. as similar, but its cheilocystidia with very thin excrescences and its non-pubescent basal disc distinguish it from our specimens. Our collections also agree with Pegler (1986), who described *M. stylobates* with basidiospores 7–10 × 2.7–4.5 µm (Q = 2.1) and cheilocystidia 20–42 × 7–13 µm, clavate, inflated, and with few to several apical protuberances. They also match Breitenbach & Kränzlin (1991), who described *M. stylobates* with basidiospores 7.2–10.8 × 3.5–4.8 µm (Q = 1.8–2.5) and cheilocystidia 28–40 × 5–11 µm, cylindrical to claviform, and with apical protuberances.

Mycena stylobates has been recorded in North America, South America, Europe, Asia, and Africa (Smith 1947, Walther et al. 2001, Desjardin et al. 2008, Franco-Molano et al. 2010, Shih et al. 2014). Desjardin et al. (2008) mentioned that the bioluminescence of *M. stylobates* is noticeable only in the mycelium; however, the observations of our studied material have shown

that the luminescent trait is also occasionally present in the pileus or in all of the basidiome, although the pileus or basidiome glows only slightly and this feature is variable among the basidiomes of distinct populations and occasionally within the same population. Desjardin et al. (2010) and Alves & Nascimento (2014) observed the same variability for *M. margarita* (Murrill) Murrill, in which some populations present bioluminescence and others lack this character.

Panellus stipticus (Bull.) P. Karst.,
Bidrag Kännedom Finlands Natur Folk 32: 96 (1879). PLATES 1D; 2J-Q

PILEUS 2–18 mm diam, rounded flabelliform to reniform, broadly convex, with an involute or inflexed margin that is crenulate or lobed; surface dull, dry, furfuraceous or appressed-squamulose, yellowish brown to yellowish white. CONTEXT thin, yellowish white; odor farinaceous. LAMELLAE decurrent, sometimes intervenose, narrowly ventricose, 0.8–1 mm broad, yellowish white, edge even, pale, crowded (16–24) with 3 series of lamellulae; edges even or fimbriate. STIPE lateral, sometimes very small or absent, 1–3 × 1–2 mm, cylindrical, narrower towards the base, solid, pubescent to tomentose, whitish. BASIDIOSPORES 3.5–5 × 2–2.5(–3) µm, (Q = 1.6–2.2), elongate, amyloid, hyaline. BASIDIA 11–18 × 3.5–5 µm, clavate or cylindrical, 2- and 4-spored with sterigmata 2–5 µm long, hyaline, inamyloid. CHEILOCYSTIDIA 22–60 × 4–7 µm, cylindrical or clavate, some rostrate or with irregular branches, hyaline, inamyloid. PLEUROCYSTIDIA absent or when the lamellae are intervenose present, 25–43 × 3–5 µm, cylindrical, some rostrate, in fascicles, hyaline, inamyloid. SUBHYMENIUM ramose to subcellular, hyaline, inamyloid. LAMELLAR TRAMA hyphae 1.5–7 µm diam, wall 0.5–3 µm thick, hyaline, inamyloid. PILEIPELLIS a cutis with hyphae 2–5 µm diam, thin or thick walled 0.5–1 µm, hyaline, inamyloid. PILEUS TRAMA hyphae 1.5–6 µm diam, thin or thick walls 0.5–3 µm, hyaline, inamyloid. STIPITIPELLIS hyphae 2–5 µm diam, smooth, thin walled, medullary hyphae, 2–7 µm diam, thin or thick walls 0.5–3 µm, cylindrical, smooth, hyaline, dextrinoid. CAULOCYSTIDIA or terminal hyphae, 25–55 × 3–5 µm, cylindrical with an acute apex, thick walled (0.8–1) µm, hyaline, inamyloid. CLAMP CONNECTIONS present in all parts of the basidiome.

BIOLUMINESCENCE—Pileus, occasionally brighter in the lamellae and in younger basidiomes. In some collections, the mycelium glows.

ECOLOGY & DISTRIBUTION—Gregarious on twigs of *Quercus* spp. in cloud forests between 1500–1600 m a.s.l.

Material studied—**MEXICO: Veracruz, Municipality of Tlalnelhuayocan**, Rancho Viejo, 23 January 2016, leg. A. Cortés-Pérez 1452, 1453; 24 September 2016, leg. Cortés-Pérez 1750; **Municipality of Coatepec**, road to Coatepec Viejo, 4 February 2017, leg. A. Cortés-Pérez 1846.

Comments—*Panellus stipticus* is a common species with a wide distribution, recorded from North America, Asia, Australasia, and Europe (Breitenbach & Kränzlin 1991, Petersen & Bermudes 1992). Nevertheless, bioluminescence has previously been cited only in populations from eastern North America (Burdsall & Miller 1975; Petersen & Bermudes 1992). While *P. stipticus* is common in western North America, collections there have not been reported as luminescent. Burdsall & Miller (1975), who revised the genus *Panellus,* described *P. stipticus* with basidiospores (3–)3.5–4.5 × 1.2–2.2 µm, elliptical to nearly allantoid in some cases, amyloid; cheilocystidia 17–45 × 3–4.5 µm, narrowly clavate, cylindrical, clavate-rostrate, fusiform to bifurcate at apex, thin-walled, hyaline; and pleurocystidia 17–40 × 3–4.5 µm, fusiform, clavate to clavate-rostrate, sometimes bifurcate at apex, thin-walled, hyaline, scattered or in dense clusters. Breitenbach & Kränzlin (1991) described *P. stipticus* without pleurocystidia; basidiospores 4–5.5 × 2.3–3.2 µm, Q=1.2–2.2; basidia 19–25 × 3.5–5 µm, clavate; and cheilocystidia 40–90 × 5–8 µm, clavate to fusiform, with or without branches. Vellinga (1995) described *P. stipticus* without pleurocystidia; basidiospores 3.5–5(–5.5) × 2–2.5(–3) µm [Q = (1.3–)1.5–1.9(–2.1)], oblong, amyloid; and cheilocystidia crowded, 38–80 × 4–8 µm, irregularly flexuous and cylindrical, sometimes branched, mostly subcapitate or broadened at the apex, sometimes with an apical excrescence, thin-walled, hyaline.

Panellus stipticus is common in the cloud forests in the state of Veracruz, but bioluminescence has not previously been observed here.

Discussion

Chew et al. (2015) reported a total of 81 bioluminescent fungi species in the world. Most of these macromycetes are distributed in tropical and subtropical zones (Desjardin et al. 2008, 2010). There are no published reports of bioluminescent fungi in Mexico despite the great biodiversity and numerous explorations made, probably because to observe the bioluminescence night explorations must be made and it is necessary to walk through the forest without lights after letting the eyes adjust to the dark. The two species mentioned here belong to the family *Mycenaceae* and, according to phylogenetic studies, they are grouped in the Mycenoid lineage (Desjardin et al. 2008). This group has the largest number of bioluminescent species, with more than 60 taxa reported (Desjardin et al. 2008, 2010; Chew et al. 2015).

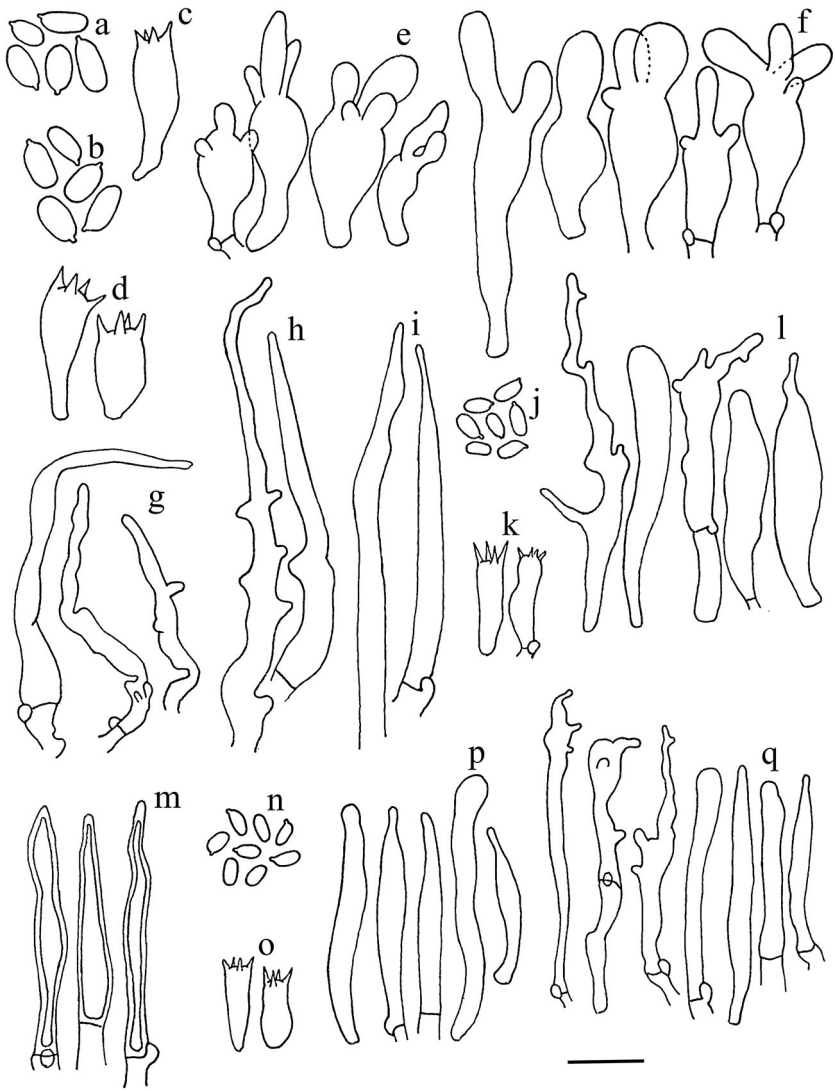

Plate 2. ***Mycena stylobates*** (A, C, E, G, I = A. Cortés-Pérez 1214; B, D, F, H = A. Cortés-Pérez & A. Rockefeller 1344). A, B: basidiospores; C, D: basidia; E, F: cheilocystidia; G, H: caulocystidia; I: basal disc cystidia. ***Panellus stipticus*** (J–M = A. Cortés-Pérez 1452; N–Q = A. Cortés-Pérez 1750). J, N: basidiospores; K, O: basidia; L, Q: cheilocystidia; M: caulocystidia; P: pleurocystidia. Scale bar = 10 µm.

Our research not only has added knowledge of bioluminescent species in Mexico but also contributes to knowledge of the distribution and ecological aspects of these species within the cloud forests.

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